MEASUREMENT OF INFLUENZA VIRUS–ANTIBODY REACTION BY QUANTITATIVE ELECTRON MICROSCOPY

LLOYD SILVERMAN, LAURENCE H. FROMMHAGEN, and DAVID GLICK

From the Division of Histochemistry, Department of Pathology, the Stanford University School of Medicine, Palo Alto, California, and the Exobiology Division, the Ames Research Center (NASA), Moffet Field, California 94304

ABSTRACT

Measurement of the weight of individual virus particles from untreated and antibody-treated populations was made by quantitative electron microscopy. The weight of antibody bound depended on the concentration of antibody in solution. One population of viruses exposed to an antibody concentration which resulted in 95% inhibition of hemagglutination showed a mass increase of 55%, corresponding to an absolute increase of 9.0×10^{-17} g in the median value. Another population, whose hemagglutination inhibition assay was 64%, showed a 39% increase in mass corresponding to an absolute median increase of 7.3×10^{-17} g. The larger viruses in each population bound a greater absolute amount of antibody than did the smaller ones, but the latter bound relatively more antibody in proportion to their mass. No cross-reactivity was found between the antibody to influenza A/PR8 and the influenza strain B/LEE. Influenza A/PR8 controls exposed to nonspecific gamma-globulin displayed a significant weight loss, at least in part owing to loss from the core, as judged from the electron micrographs.

INTRODUCTION

A method of quantitative electron microscopy that permits the measurement of mass to 10^{-18} g has been developed through the work of Zeitler and Bahr (8), Lenz (6), Hall (4), Burge and Sylvester (2), and others. In this study the method of Zeitler and Bahr was used with minor modifications to quantitate the reaction, on an intact biological object, between a known antigen and its antibody. The influenza virus was chosen as a model whose physical and serologic properties have been intensively studied. The sensitivity of previous methods used to quantitate antigen–antibody reactions has been given by Haurowitz (5) as 12–100 μg antibody by the microprecipitation technique and 0.03 μg both by passive hemagglutination and Coombs antiglobulin tests.

MATERIALS AND METHODS

Preparation of Virus

Viruses of the influenza strains A/PR8 and B/LEE were grown in embryonated eggs and purified by adsorption to, and elution from, human type "O" cells; this group of viruses will be referred to as population A. A second lot of A/PR8 virus, prepared similarly, was banded in a sucrose density gradient (12–42%) to produce a more uniform population, referred to as population B (Fig. 1).

TABLE I

Reaction of Influenza A/PR 8 with Specific and Nonspecific Antibodies and Correlation of Antibody Uptake with Hemagglutination Inhibition Assay

Virus population	No. of Particles	Mean Mass × 10^{-17} g	SD	Virus/globulin	HI assay*	Mass change	*P*
				mg	%	%	
A A/PR8 lot 67							
Untreated	95	16	5.3				
Anti-V-PR8	119	25	6.1		95	+55	<0.001
B A/PR8 lot 72							
Untreated	118	19	3.8				
Plus anti-V-PR8	104	26	3.9	0.03/0.36	64	+39	<0.001
Plus nonspecific‡	104	16	3.8	0.03/0.40	0	−16	<0.001
C A/PR8 lot 72							
Untreated	95	16	3.2				
Plus nonspecific only	100	12	2.3			−25	<0.001
Plus nonspecific and Sephadex	97	10	2.5			−38	<0.001
D B/LEE							
Untreated	156	16	3.2				
Plus nonspecific	132	16	3.2			0	1.0
Plus anti-V-PR8 and Sephadex	93	15	3.2			−6.3	<0.001

* Hemagglutination inhibition assay

‡ Nonspecific globulin

Preparation of Antibody and Reaction with Virus

Antibody was produced in guinea pigs to the "V" antigen, i.e. the specific strain antigen of the virus, which had been prepared by the ether method (7). Gamma globulin was isolated, by the rivanol procedure, from the hyperimmune serum and excess rivanol was removed by passage through Sephadex G-50 (3). This globulin was used as specific antibody, "anti-V-PR8." Nonspecific antibody was prepared as the ethanol fraction II of normal guinea pig gamma-globulin.

Both populations of influenza A/PR8 and one of influenza B/LEE were reacted with anti-V-PR8, and unbound globulin was almost totally removed by three sedimentations of the virus-antibody complexes at 30,000 *g* followed by resuspension in 0.1 M phosphate buffer, pH 7.0. Additional samples from the three populations were reacted with nonspecific antibody and were washed as before. In an effort to obviate the significant loss of mass observed after the latter procedure, further samples of A/PR8 and B/LEE were reacted with nonspecific antibody, and all unreacted protein was removed from the virus on Sephadex G-200.

Antibody nitrogen was measured by a micro-Kjehldahl procedure. Hemagglutination-inhibition assay, with human type O cells, was employed as an independent estimate of antibody uptake. The test employed four hemagglutinating units of antigen and dilution volumes of 0.25 ml (Table I).

Measurement of Mass

Formvar-carbon-coated grids were placed on droplets of virus suspension, washed three times, 5 min each, on droplets of 0.1 M ammonium acetate and then were dried in air. Polystyrene latex spheres, approximately 1260 A in diameter, were sprayed on the grids for use as focusing aids and weight standards. Magnifications were measured by means of a diffraction grating replica (21,000 lines/cm). Photographs were taken at a magnification of approximately 50,000 times with a Hitachi HU 11-A electron microscope equipped with a special photometer-exposure timer which permitted exact exposures necessary to produce the required background densities. Other operating conditions included an accelerating voltage of 50 kv; objective aperture, 30 μ; liquid nitrogen in the specimen-stage cold trap; and the use of the high-contrast specimen holder. Photographs were made as close to focus as possible to obviate the effects of phase contrast which had a significant influence on the measurement of mass of these small particles.

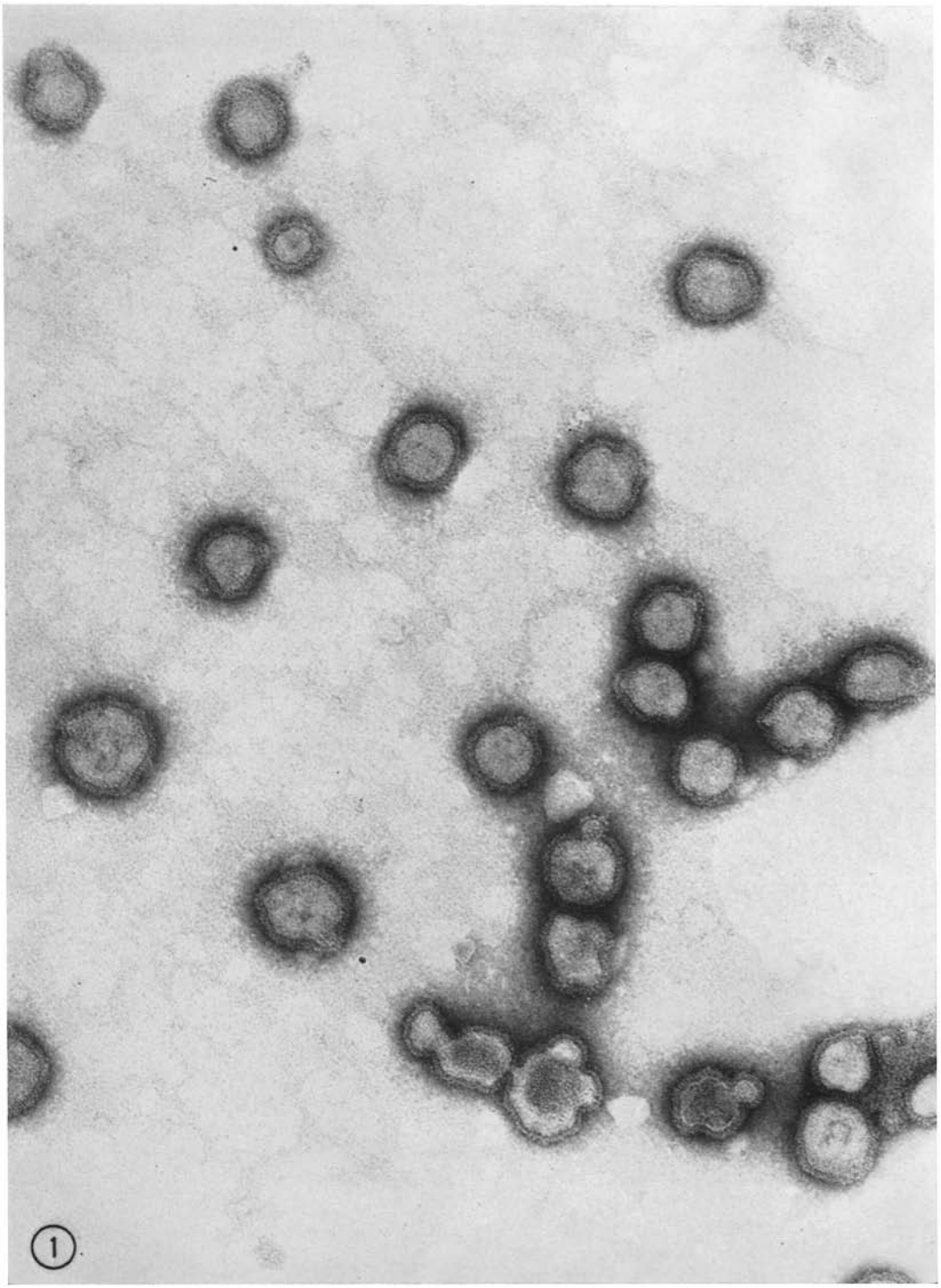

FIGURE 1 Influenza A/PR8 virus, negatively stained with 2% phosphotungstic acid, pH 6.4. A range of sizes is evident in the population. Distortions of some of the virions were not seen in unstained preparations. × 100,000.

The negative images of the virus particles (Figs. 2, 3) and the latex spheres were scanned in a Jarrell-Ash recording microdensitometer and the areas under the resulting curves were measured with a compensating polar planimeter. The mass of each sphere was calculated from its known density (1.05) and its radius, which was derived from the measured image. A factor relating area under the curves to mass was derived from the spheres, and the mass of individual virions was determined. Prolonged exposure to the beam, even with the use of liquid nitrogen, led to contamination of the spheres which resulted in progressive decrease in the factor.

Each population was plotted linearly on probability paper as percent integral frequency against logarithm of weight (1).

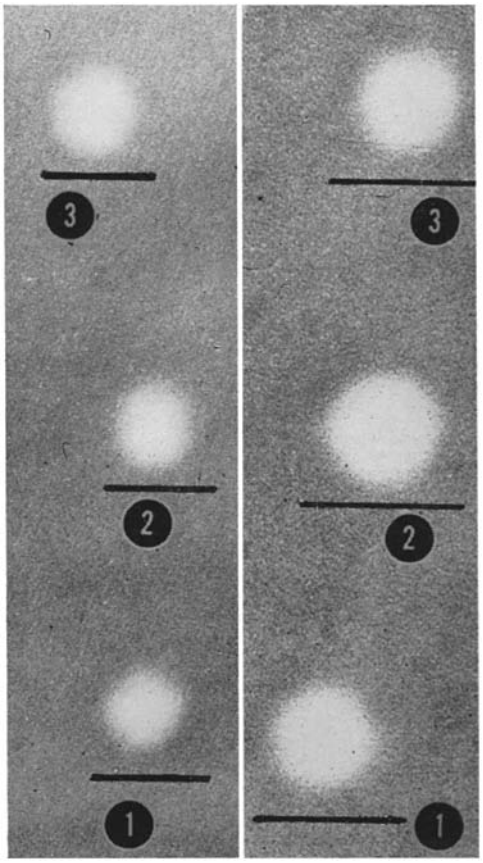

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FIGURE 2 Influenza A/PR8 population B, untreated, negative image used for densitometry (see Table I). (Line and number under image of each virus particle used as guides in densitometry.) × 100,000.

FIGURE 3 Influenza A/PR8 population B, treated with anti-V-PR8, negative image (see Table I). × 100,000.

RESULTS

A representative sample of influenza A/PR8 (population A) is seen to contain virions of various sizes (Fig. 1). Populations A and B, untreated and and treated with anti-V-PR8 (Table I), are represented by the lines in Fig. 4 whose slopes represent standard deviation (median at 50% integral frequency). The lines have been fitted by the method of least squares. The steeper slope of population B compared to population A reflects the more uniform population achieved by gradient centrifugation.

The convergence of the lines for each population indicates that the smaller viruses in each population bind relatively more antibody in proportion to their mass than the large viruses, although the latter bind larger absolute amounts of antibody.

The mass increase appears to vary with the concentration of antibody used, and correlates with inhibition of hemagglutination (Table I).

The loss of mass caused by exposure to nonspecific antibody was reproducible in both lots of A/PR8 virus tested. Influenza B/LEE was comparatively resistant to such loss.

The parallelism of the lines for these populations (Fig. 5) indicates that the loss of mass was a constant fraction of the original weight of the virus; part of the loss occurred apparently from the core (Fig. 6). The treated virus preparation was passed through a Sephadex G-200 column to remove excess protein more completely, but this did not prevent the loss (Table I). Viruses exposed to specific antibody did not demonstrate this change (Fig. 7).

Anti-V-PR8 was not bound by influenza B/LEE (Table I).

DISCUSSION

This method has several advantages for the measurement of antigen–antibody reactions of biological particles. Only small numbers of particles are required and the distribution (not simply the mean) of mass in the population can be assessed. Thus, differences in behavior of particles of differing sizes are detectable. In addition, available or biologically active sites can be studied without the need for separating the antigen from the particle.

The finding that smaller viruses in the population take up relatively more antibody in proportion to their mass than do the larger ones may be related to their surface-to-volume ratios. Decrease

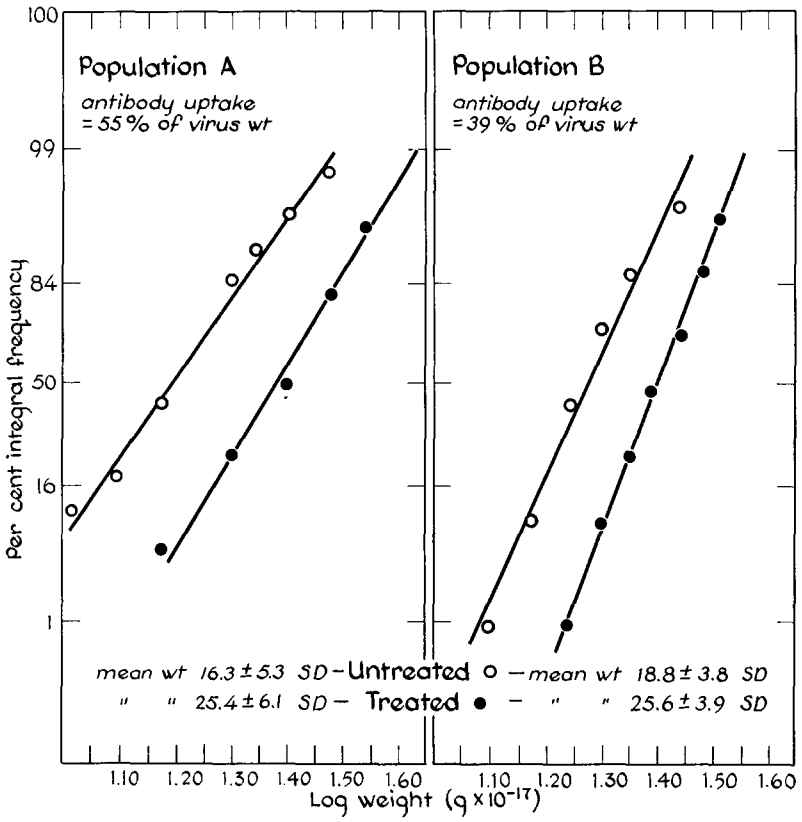

FIGURE 4 The distribution of particle weights before and after the addition of antibody in each of two populations. The steeper slope of the lines for population B reflects the greater uniformity of the population. The larger increment between the lines in population A reflects exposure of the population to a higher concentration of antibody.

in mass after exposure to nonspecific globulin is a constant fraction of the original virus mass and seems to involve loss of substance from core as well as surface. The mechanism of the loss has not been elucidated. However, it was determined that unreacted gamma-globulin could be removed more conveniently and efficiently by passage through a Sephadex G-200 column than by the sedimentation method usually employed for this purpose.

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For References, see page 66.

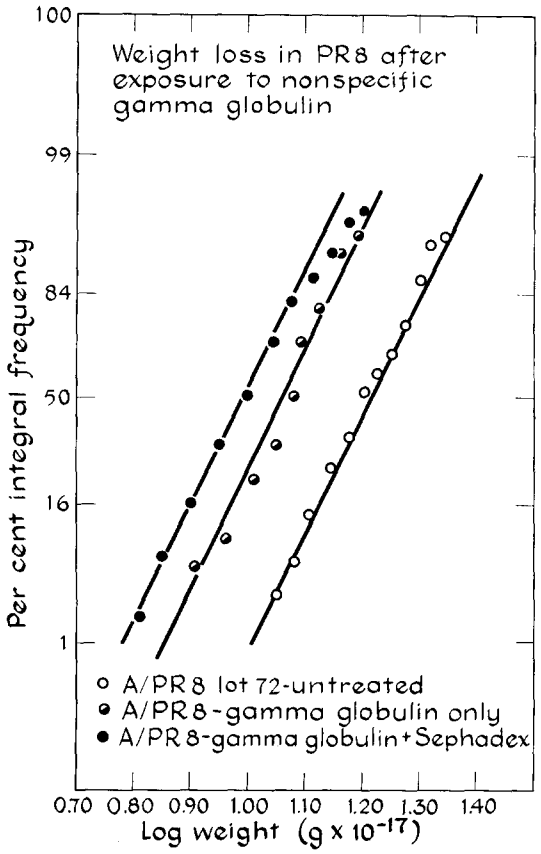

FIGURE 5 The effect on influenza A/PR8 of exposure to nonspecific gamma-globulin. This loss of mass was not reflected in a change in the hemagglutination inhibition assay.

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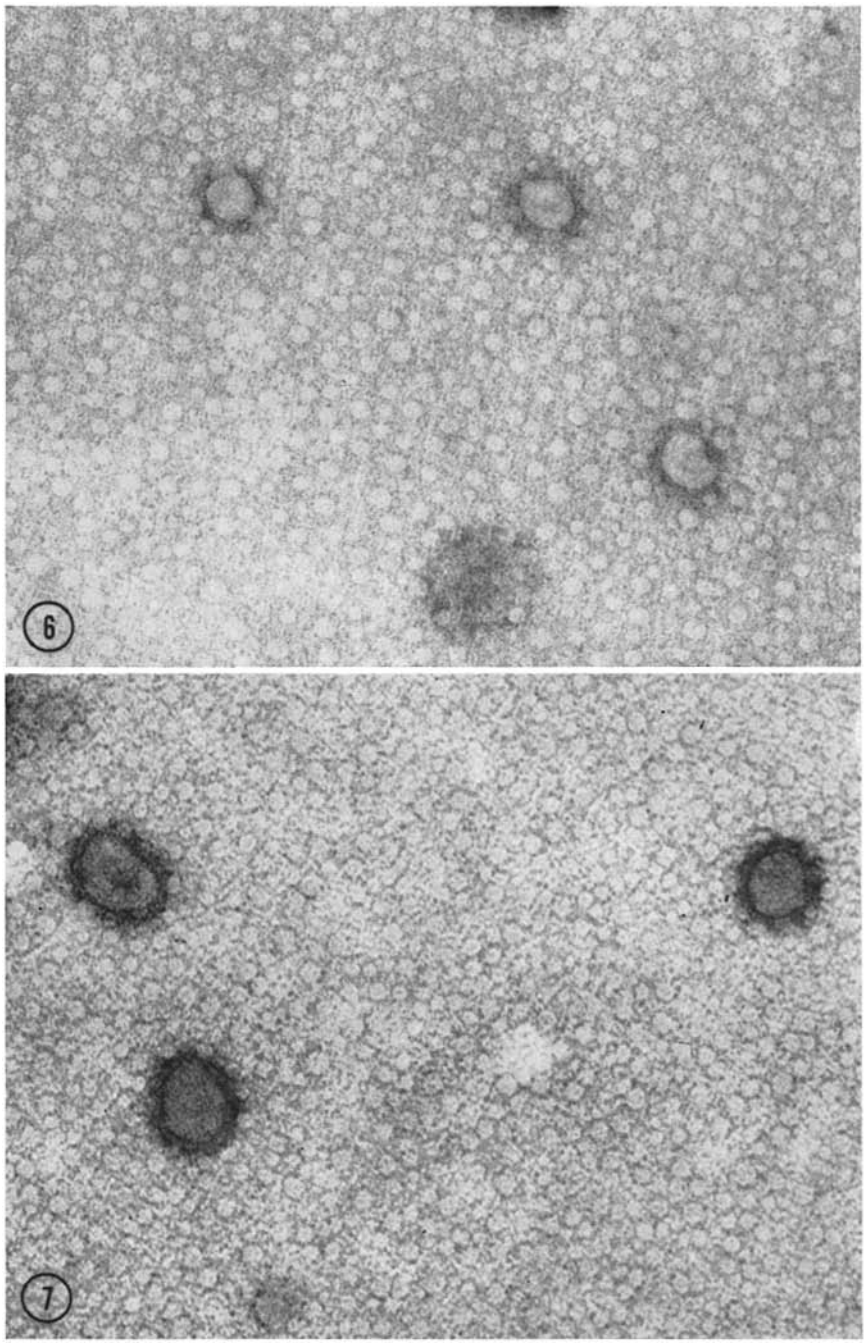

FIGURE 6 Influenza A/PR8, population B, treated with nonspecific gamma-globulin, negatively stained. × 100,000.

FIGURE 7 Influenza A/PR8, population B, treated with anti-V-PR8, negatively stained. × 100,000.